

# ***The Relationship Between Lateral-Root Development and Spread of *Pisolithus tinctorius* Ectomycorrhizae After Planting of Container-Grown Loblolly Pine Seedlings***

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**ABSTRACT.** Container-grown loblolly pine (*Pinus taeda* L.) seedlings with *Pisolithus* ectomycorrhizae were planted in microplots containing fumigated or nonfumigated forest soil. At 4-week intervals during a 20-week period after planting, seedlings were excavated to determine the pattern of lateral root egress and spread of *Pisolithus* ectomycorrhizae on laterals. Between 75 and 80 percent of the laterals egressed from the bottom of the plugs in both fumigated and nonfumigated soil. Hyphal strands of *Pisolithus* spread from the plugs and developed ectomycorrhizae at greater distances from the plugs on lateral roots of seedlings in fumigated soil than on seedlings in nonfumigated soil. The results indicated that root configuration of container-grown loblolly seedlings initially influences lateral root egress (first 20 weeks) after planting. Soil microbial associates, i.e., native symbionts and pathogenic fungi, of the more vertically egressed roots in nonfumigated soil apparently do not favor development of *Pisolithus* ectomycorrhizae. *FOREST SCI.* 29:519-526.

**ADDITIONAL KEY WORDS.** *Pinus taeda*, root configuration.

INOCULATING CONTAINER-GROWN PINE SEEDLINGS with *Pisolithus tinctorius* (Pt) (Pers.) Coker & Couch before planting improves their growth and survival after planting on certain adverse sites (Ruehle 1980b, Berry<sup>1</sup>). However, container-grown seedlings with Pt ectomycorrhizae planted on routine sites often fail to show improved performance over seedlings colonized with naturally occurring ectomycorrhizal fungi (Ruehle and others 1981, Ruehle and Brendemuehl 1981, Marx and Barnett 1974), nor does their performance compare well with that of bare-root seedlings with Pt ectomycorrhizae planted on routine sites (Marx and others 1977, Ruehle and others 1981).

Containers characteristically modify pine root systems (Bilan and others 1978). While the seedlings are very young, the container imprints its configuration on the root system and the major lateral roots tend to lie parallel to the main root

<sup>1</sup> Berry, C. R. Survival and growth of pitch, loblolly, and pitch  $\times$  loblolly pine hybrid seedlings with *Pisolithus* ectomycorrhizae on coal spoils in Alabama and Tennessee. Southeastern Forest Experiment Station, Athens, Ga. (In press, *Journal of Environmental Quality*.)

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for the first 10 or 15 cm of their length rather than spreading horizontally, as they do with bare-root seedlings. With the parallel configuration, new lateral roots initially extend more or less vertically into the soil to encounter a microenvironment and soil microorganisms less favorable to the development of *Pt* ectomycorrhizae than those found nearer the soil surface (Ruehle and others 1981).

A study of container-grown loblolly pine (*Pinus taeda* L.) was established with seedlings planted in both fumigated and nonfumigated soils to determine how root extension after planting, as well as indigenous fungal symbionts and associated microflora, affect spread of *Pt* from root plugs to newly emerging feeder roots.

## MATERIALS AND METHODS

Mycelial inoculum of *Pt* (isolate 250) was produced, leached, and dried as previously reported (Marx and Bryan 1975, Marx and Rowan 1981). A growing medium was prepared by mixing screened peat moss and horticultural grade vermiculite at a rate of 1:1 v/v. Inoculum (ca 50 percent moisture) was mixed with the growing medium at a rate of 1:10 v/v. Control seedlings received no inoculum. Hillson Rootainers® (Spencer-Lemaire Industries, Ltd., Edmonton, Alberta, Canada) were filled (ca 125 cm<sup>3</sup> growing medium/cavity), watered with a dilute wetting agent (Tween® 20), and seeded with stratified loblolly pine seed (mixed lot collected in South Carolina).

Three weeks after germination, seedlings were thinned to one per cavity and drenched with a modified nutrient solution (Ruehle 1980a) plus benomyl fungicide (0.4 g/l). All seedlings were fertilized two more times at 4-week intervals with a commercially available water soluble fertilizer (NPK at 500 µg/ml) and applied to saturation.

After 14 weeks, all seedlings were visually rated for ectomycorrhiza development. Two hundred and twenty seedlings well colonized with *Pt* were selected from 600 inoculated seedlings, and 52 comparable control seedlings were selected from 250 noninoculated seedlings. From these two groups 20 *Pt* seedlings and 12 control seedlings were selected at random, carefully rinsed free of growing medium and visually assessed for mycorrhiza development (Marx and Bryan 1975). *Pt* seedlings averaged 28 (±13.1) percent *Pt* and 14 (±9.0) percent other fungi, mostly *Thelephora terrestris*; control seedlings averaged 24 (±13.4) percent other fungi.

Sixty wood-frame microplots (122 × 61 × 40 cm) were constructed on level ground in 5 blocks of 12 adjoining microplots each. Microplots and adjacent soil were covered with clear polyethylene and fumigated with methyl bromide (MC-2) at recommended rates. Twenty-four cubic meters of sandy loam were collected from a forest site in the Oconee National Forest, placed on a concrete slab and tilled to remove stones and large root fragments. A composite soil sample taken from this pile was processed and analyzed.<sup>2</sup> The soil was air-dried at room temperature and extracted with a double acid solution (0.05 N HCl + 0.025 N H<sub>2</sub>SO<sub>4</sub>). Phosphorus (P) was determined colorimetrically and cations by atomic absorption. Total N was determined by Kjeldahl, organic matter by wet oxidation chromic acid digestion, and pH by glass electrode in a soil paste. In µg/g, the soil averaged available P = 19, exchangeable K = 71, exchangeable Ca = 317, exchangeable Mg = 41, and NO<sub>3</sub>-N = 580. The soil was 80 percent sand, 9 percent silt, and 11 percent clay; it contained 1.7 percent organic matter and had a pH of 5.6. Half of the soil was covered with clear polyethylene, fumigated with methyl bromide (MC-2) for 48 hours and vented. Five soil samples were also randomly collected from this pile before and after fumigation and assayed for *Pythium* and *Phy-*

<sup>2</sup> Analyses performed by Carol G. Wells, USDA Forest Service, Forestry Sciences Laboratory, Southeastern Forest Experiment Station, Research Triangle Park, N.C.

*tophthora* spp. using modified Kerr's medium (Hendrix and Kuhlman 1965) and for nematodes using a centrifugal-flotation technique (Jenkins 1964). Before fumigation there was an average of 9 propagules of *Pythium* spp. per gram of soil and 19 *Criconeimoides* sp. and 200 saprophytic nematodes per 500 cc of soil. After fumigation no fungi or nematodes were found. Half of the microplots were filled with fumigated soil and half with nonfumigated soil. The soil in each plot was well packed and watered to saturation. Treatments were assigned to plots in a randomized block design of five replicates.

On May 28, 1981, four seedlings were hand-planted (evenly spaced in a single row) in each plot with a bullet-shaped planting tool. Height and root-collar diameter of each seedling were measured. All plots were watered at planting time. No fertilizer or additional water was added during the course of the study.

Rainfall was recorded at a weather station (NOAA, Athens) approximately 3 km from the study site. After the planting date (May 28) until the study was terminated (October 16) rainfall was as follows:

Month	No. of rainfalls	Total mm
May	1	16
June	2	38
July	5	59
August	5	25
September	3	26
October	1	13

Four weeks after planting and every 4 weeks thereafter, all Pt seedlings were carefully excavated by hand from one fumigated plot and one nonfumigated plot within each block. At the fifth and last sampling period (20 weeks), seedlings from the two control plots in each block were also excavated. When excavated, each root plug was visually divided into three horizontal zones by using a modification of a procedure developed by Rischbieter (1978): A = top 4 cm, B = middle 4 cm, C = bottom curved portion 2.75 cm. The number of lateral roots emerging from each zone, the length of each lateral root, the number of Pt ectomycorrhizae present, and the greatest distance from the plug that Pt ectomycorrhizae had developed were recorded. The height and root-collar diameter of each seedling were measured. At the last sampling period, the fresh weight of top and root also were recorded for both Pt and control seedlings. The data were analyzed by analysis of variance and significant means were separated according to Duncan's Multiple Range Test or Student's *t*-test ( $P = 0.05$ ).

## RESULTS

The pattern set for lateral root growth out from the plug zones at the first sampling period (4 weeks) (Fig. 1) was still evident at the fifth sampling period (20 weeks). Seventy-five to eighty percent of the laterals egressed from zone C (Fig. 2). Among Pt inoculated seedlings soil treatment did not affect root development from zones A or B during the 20-week period (Table 1). In fumigated soil, there were significantly more roots growing out from zone C. Among the control seedlings, significantly more roots grew out from zones B and C on seedlings in fumigated soil than on seedlings in nonfumigated soil (Table 1).

Pt hyphal strands spread from the plugs and developed ectomycorrhizae farther out on laterals in fumigated soil than in nonfumigated soil. At every sampling period, Pt ectomycorrhizae were found at approximately twice the distance from the plug on seedlings growing in fumigated soil than on those growing in non-

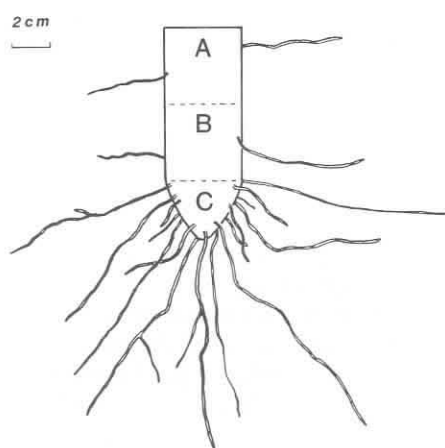


FIGURE 1. Typical pattern of egression of lateral roots of loblolly pine from root plugs 4 weeks after planting in fumigated soil.

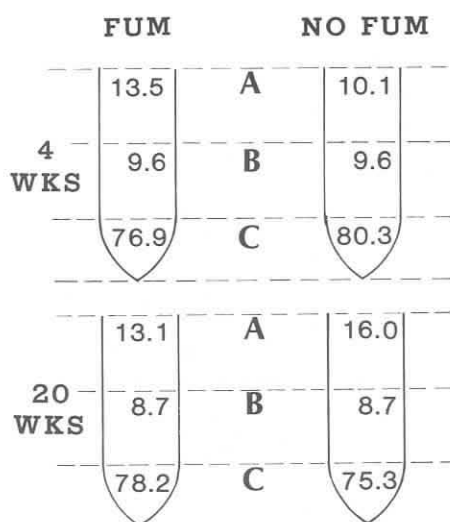


FIGURE 2. Mean percentage of total roots egressing from root plugs from each of the three zones at 4 and 20 weeks in fumigated and nonfumigated soil. Means calculated on four seedlings per plot for each of five replicates.

fumigated soil (Fig. 3). By the fourth sampling period (16 weeks), *Pt* ectomycorrhizae had developed on laterals out to the edge of the plots containing fumigated soil, making it difficult to measure and determine the spread of *Pt* after this period.

Seedling growth was significantly greater in fumigated soil than in nonfumigated soil (Table 2). Mycorrhizal treatment had no effect on height, root-collar diameter, or top fresh weight, regardless of soil treatment. *Pt* seedlings, however, did have significantly greater fresh root weight than control seedlings in both fumigated and nonfumigated soil.

At the last sampling period (20 weeks) many laterals were broken and *Pt* ectomycorrhizae were stripped off because numerous lateral roots had intermixed

TABLE 1. Mean number of lateral roots emerging from horizontal plug zones of container-grown loblolly pine seedlings planted in fumigated and nonfumigated soil.

Zone and soil treatment	Sampling period (wk)					Control (20 wk)
	4	8	12	16	20	
Zone A						
Fumigated	2.8	3.1	3.2	4.8	3.8	3.3
Nonfumigated	2.1	3.0	2.1	3.6	3.5	2.5
Zone B						
Fumigated	2.0	1.9	2.6	2.1	2.5	2.0*
Nonfumigated	2.0	1.5	1.7	1.2	1.9	1.1*
Zone C						
Fumigated	16.0	18.5*	21.0	21.8*	22.6*	21.6*
Nonfumigated	16.7	12.4*	17.4	17.8*	16.5*	15.3*

\* = significantly different ( $P = 0.05$ ) according to Student's *t*-test for paired means.

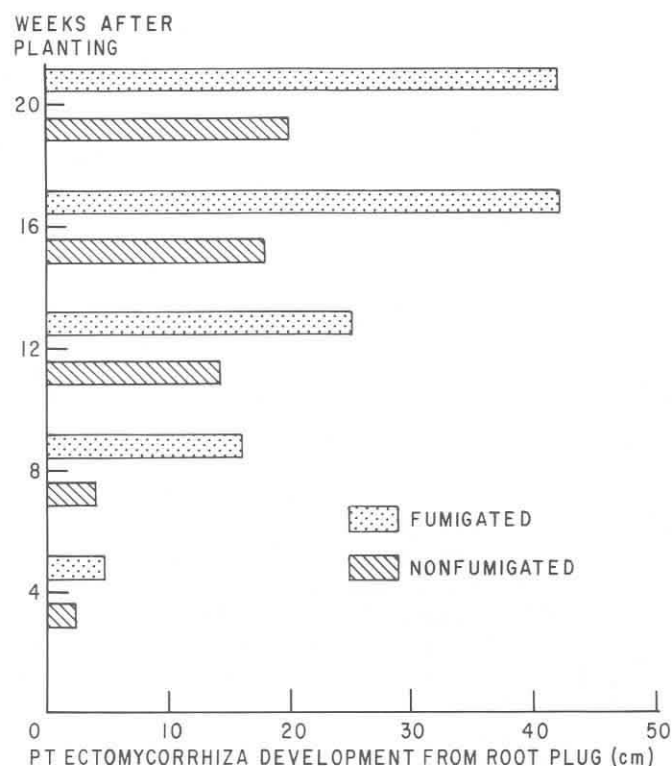


FIGURE 3. Maximum distances *Pt* ectomycorrhizae were observed on loblolly pine laterals egressed from plugs in both fumigated and nonfumigated soil at each sampling period. Based on means of four seedlings per plot for each of five replicates.

with roots of adjoining seedlings and had extended around the sides of the plots. The recorded number of *Pt* ectomycorrhizae at this period was actually an underestimate of the total number developed. Since the numbers lost were assumed to be the same for seedlings excavated from both fumigated and nonfumigated soil, the recorded number still provided relative values useful for comparing groups. Lateral root growth and development of *Pt* ectomycorrhizae were evaluated on the data from the fourth sampling period (16 weeks). There was no

TABLE 2. Growth of *Pisolithus* (*Pt*) inoculated and noninoculated (*NI*) loblolly pine seedlings under two soil treatments after 20 weeks.

Soil treatment and inoculation	Height	Root-collar diameter	Fresh weight	
			Top	Root
Fumigated soil	cm	mm	g	g
Pt	45.9 a	15.0 a	161.3 a	112.3 a
NI	47.1 a	13.9 a	160.6 a	82.2 b
Nonfumigated soil				
Pt	31.8 b	10.8 b	64.8 b	75.1 b
NI	28.7 b	10.7 b	58.1 b	44.0 c

Values are means of four seedlings per plot for each of five replicates. Values in a column followed by the same letter are not significantly different at  $P = 0.05$ .

TABLE 3. Development of *Pisolithus ectomycorrhizae* on lateral root growth of container-grown loblolly pine seedlings after 16 weeks in fumigated and nonfumigated soil.

Soil treatment	Number laterals with Pt and total laterals	Percentage of laterals with Pt	Total length lateral roots (cm)	Total number Pt ectomycorrhizae	Pt ectomycorrhizae/cm lateral root <sup>1</sup>
Fumigated	27.0/28.8	94.3 ± 2.6	936.9 ± 77.5	1,041 ± 24	1.2 ± 0.2
Nonfumigated	9.5/22.6	42.8 ± 14.6	551.4 ± 164.1	131 ± 100	0.5 ± 0.2

<sup>1</sup> Only those lateral roots with *Pt* ectomycorrhizae were used to calculate this ratio.

Values are means and standard deviations based on summation of four seedlings per plot for each of five replicates.

difference in number of short roots per centimeter of lateral root (ca 2 per centimeter on sections supporting short roots) in fumigated and nonfumigated soil. Over 90 percent of the egress laterals on seedlings growing in fumigated soil had *Pt* ectomycorrhizae, while those on seedlings growing in nonfumigated soil had less than 50 percent (Table 3). The total length of laterals on seedlings in fumigated soil was almost double that on seedlings in nonfumigated soil. Seedlings in fumigated soil had eight times more *Pt* ectomycorrhizae than seedlings in nonfumigated soil (Table 3). Also, when the ratio of *Pt* ectomycorrhizae to length of laterals was calculated, seedlings in fumigated soil had double the number per centimeter of lateral root than those seedlings in nonfumigated soil.

In over half of the fumigated and nonfumigated plots at the third, fourth, and fifth sampling periods, hyphal strands were observed along the surface of lateral roots and extending away from the roots. At the fifth sampling period some intact hyphal strands were found more than 30 cm from the plugs.

#### DISCUSSION

Lateral root development in the lower portion of the plug resulted from the distribution of root tips at the time of outplanting, a feature also reported by Long (1978). This orientation is considered desirable for container-grown Douglas-fir because many active root tips are ready to penetrate to deeper layers of soil, and thus develop a more favorable internal water balance throughout the growing season (Hahn and Hutchison 1978). However, if container-grown southern pines inoculated with specific mycorrhizal symbionts are employed in routine reforestation where the primary objective is to obtain good spread of the symbiotic fungus to egressed lateral roots, this orientation may not be desirable. Our results revealed that *Pt* spread poorly and formed few *Pt* ectomycorrhizae on lateral roots egressed from the bottom of plugs planted in nonfumigated soil.

Soil fumigation removed the biological factors that inhibit spread of *Pt* hyphae out of the plug and development of *Pt* ectomycorrhizae on egressed lateral roots. During excavation of seedlings from nonfumigated plots at the third (12 weeks) and fourth (16 weeks) sampling periods, hyphal strands of *Pt* traversed the surface of egressed lateral roots more than 20 cm from the plug. However, the forest soil used in this study had indigenous ectomycorrhizal fungi (mostly *Thelephora terrestris*) and most of the short roots in these areas were infected with them. In nonfumigated plots, the root and mycorrhiza patterns that emerged were as follows: the lateral roots extended from the plug, short roots emerged and became infected by indigenous fungi and formed ectomycorrhizae; when the *Pt* hyphal



strands grew out along the surface of the lateral roots, they encountered few unoccupied infection sites. The pattern that developed in fumigated plots was different. Soil fumigation eliminated the indigenous symbiotic fungi, the lateral roots extended out from the plug, short roots emerged, but remained noninfected; when the *Pt* hyphal strands spread out along the roots, they infected the short roots and formed ectomycorrhizae. This resulted in greater numbers of *Pt* ectomycorrhizae on roots growing in fumigated soil (Table 3).

Fumigation may have eliminated, or reduced to very low numbers, propagules of soil-borne pathogenic fungi capable of lowering the rate and degree of lateral root development, thus lowering the numbers of short roots available for infection by mycorrhizal fungi. The soil was not assayed for propagules of fungal pathogens, but several segments of lateral roots on over half of the seedlings excavated from nonfumigated plots showed thickening and dark-brown to black surface discoloration—symptoms of some type of low-level pathogenesis.

Fumigation markedly affected factors influencing seedling growth. After 20 weeks, growth differed significantly between seedlings in fumigated and nonfumigated soil (Table 2). However, under conditions of this study, inoculation with *Pt* affected seedling growth little. This is in agreement with past work on reforestation sites where growth differences caused by *Pt* were rarely seen during the first year; most differences were observed after two growing seasons (Marx and others 1977, Ruehle and others 1981).

To obtain benefits from inoculation of container-grown pines produced for outplanting on routine forest sites, some means of controlling root morphogenesis might be developed. The ridges and grooves of roottrainers eliminate spiralling of lateral roots, but still press the root system into a container-size root plug with lateral roots oriented vertically downward. After planting, this root configuration results in a mostly downward early root egress from the plug. Changing lateral root morphogenesis in the plugs to permit a more horizontal egress of laterals after planting by coating the container walls with chemicals that inhibit root extension (Burdett 1978, McDonald 1981) might solve this problem.

Additional research is planned to compare container-grown and bare-root pine seedlings to answer the question of root egress after planting and how this affects the spread of *Pt* to newly formed roots. Such investigations should provide clues to explain the differences in growth performance between the two types of planting stock used in past field trials.

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